



Xylem anatomical responses to climate variability in Himalayan birch trees at one of the world's highest forest limit

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ABSTRACT

The Himalayas is one of the most ecologically sensitive and fragile areas in the world. The climate of the region is dominated by the monsoon seasonality, with typical dry winters and abundant summer precipitations. Here, forest vegetation spreads up to the world's highest elevations, where cold temperatures and early spring droughts represent the main limiting factors for growth. In this study, we applied a dendro-anatomical approach to assess xylem trait chronologies and their association to local climate variability in the diffused broadleaved *Betula utilis* D Don close to the treeline (above 3900 m a.s.l.). We measured tree-ring width on increment cores from 73 trees. On seven cores, we prepared 12 μm thick sections, which were analysed with ROXAS for the assessment of ring-based xylem parameters. We then built up the corresponding trait chronologies and analyzed them against the time series of monthly temperatures and precipitations. Mean ring width (MRW), mean vessel area (MCA_V) and ring-specific hydraulic conductivity (Kr) positively correlated with summer temperatures in the previous and current year. In addition, MCA_V was significantly associated with March precipitations. Instead, fibres' area showed a widely negative correlation only with temperatures during the previous and current season suggesting that fibres get narrower when vessels are wider. These results revealed that Himalayan birch is well adapted to the monsoon seasonality and is responding positively to atmospheric warming, thus suggesting the potential for this species to further expand in altitude under the forecasted climate change scenarios.

1. Introduction

The Himalayan region is one of the world's major climate change hotspots. Mean annual temperature is increasing faster than the mean global increase (IPCC, 2014), up to a rate of $0.06\text{--}0.1\text{ }^{\circ}\text{C yr}^{-1}$ since the mid-1970s (Qi et al., 2013; Shrestha et al., 2012). With the rapid warming, the melting of glaciers and the altitudinal advance of forests may trigger an additional positive feedback mostly related to reduced surface albedo (Winton, 2006). Given the mean tropospheric temperature lapse rate of $-0.56\text{ }^{\circ}\text{C}$ every 100 m of altitude, it follows that similar environmental conditions setting the altitudinal limit of forest expansion only 40 years ago, would nowadays potentially occur between 400 and 700 m higher up.

High altitude and high latitude forest limits are usually set by low temperatures, which directly limit the metabolic activity of synthesizing structural carbon compounds (i.e., source-sink limitation), and indirectly reduce the length of the vegetative season in the case of seasonality (Körner, 2012). In general, the tree growth form in the cold environment seems to be set by the thermal threshold of $\sim 6.7\text{ }^{\circ}\text{C}$ in

mean ground temperature during the growing season (Körner and Paulsen, 2004). However, vegetation at high altitudes in the Himalayas is not only experiencing a rapid release of temperature limitations to growth, but also a potential increase in the occurrence of water stress conditions at the beginning of the growing season. In fact, the Himalayan climate is strongly influenced by the monsoon, with abundant summer precipitations (approximately from June to September) and dryer conditions during winter. At forest limit elevations the monsoon season is shorter (i.e., it starts later and ends earlier), and during the dryer winter season, snow can accumulate on the ground (Salerno et al., 2015; Schickhoff et al., 2015). An important effect of climate warming is that higher temperatures would both turn part of the snowy precipitations into rain and anticipate the snowmelt (Ming et al., 2015). A reduced snow pack that melts earlier could potentially produce a strong ecological pressure on the high altitude vegetation due to the resulting lower soil water availability at the beginning of the growing season (Körner, 2012).

In the face of the rapid changes in climate, a comprehensive knowledge of the ecology of Himalayan high-altitude trees is very

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important to better understand and predict the specific responses to local climate variability and the overall dynamics of forest vegetation that could occur in the near future at the world's highest altitudinal ranges. The highest forest belts in the Eastern Nepali Himalaya, at approximately 4100 m a.s.l., are mainly formed by *Abies spectabilis* D. Don, together with broadleaved Himalayan birch (*Betula utilis* D. Don) and tree-like *Rhododendron* sp., *Sorbus microphylla*, *Acer campbelli* (Zobel and Singh, 1997).

The few studies conducted on climate-growth relationships in Himalayan birch mainly focused on the analysis of ring widths. This species growth is sensitive to the pre-monsoon climate, with ring width being positively (negatively) correlated with spring precipitations (temperature) (Bhattacharyya et al., 2006; Dawadi et al., 2013; Liang et al., 2014). In recent years, a novel sub-discipline of dendrochronology, namely dendro-anatomy (or quantitative wood anatomy) has been attracting the interest of the scientific community. The variability of the different xylem anatomical traits encodes specific imprints of climatic effects not visible with ordinary approaches, with potentially relevant effects for the tree physiological processes (Fonti et al., 2010; Hacke and Sperry, 2001). The first attempts to study the effects of climate on wood anatomical traits in broadleaved species were carried out by analyzing the interannual variation of vessel lumen area (Fonti and García-González, 2004; García-González and Fonti, 2008; González and Eckstein, 2003; Souto-Herrero et al., 2017).

More recently, important advancements in sample preparation and image analysis allowed to increase by several orders of magnitude the amount of anatomical data obtainable from a single tree ring (von Arx and Carrer, 2014). On the one hand, this facilitated the build-up of centuries-long chronologies of cell parameters (Carrer et al., 2015; Castagneri et al., 2015); on the other, it opened the possibility to explore other anatomical traits rather than just the tracheid/vessel size. For instance, the intra-annual variability of vessel/tracheid lumen area or cell wall thickness can be more easily analyzed for retrospective analyses of cambium phenology (Carrer et al., 2017; Castagneri et al., 2017). Among other overlooked xylem elements that can be analysed thanks to the new methodologies, fibres could be considered for the potential climate information. In particular, fibres' density supposedly plays a relevant role in the resistance to drought-induced cavitation of the whole xylem transport system (Jacobsen et al., 2005), and can also modify the overall water transport capacity and wood strength in several species (De Micco et al., 2016).

This paper presents a dendro-anatomical study on the association of different xylem anatomical traits with temperature and precipitations in Himalayan birch close to its altitudinal limit in the Kumbhu Valley (Sagarmatha – Everest National Park), Eastern Himalaya. We compiled the first regional century-long chronology of vessel and fibre anatomy and correlated it with the available meteorological data to disentangle the climate information potentially encoded in different xylem anatomical traits. With this approach, we aimed at improving the current knowledge on the ecology of Himalayan birch to better understand its acclimation/adaptation potential at one of the world's climate change hotspots.

2. Materials and methods

2.1. Study area and climate

The study area is in the Sagarmatha (Everest) National Park, North-Eastern Nepal (Fig. 1). The target species in this study is *Betula utilis* D. Don, which is distributed from Afghanistan to Nepal in the Himalayas (Bobrowski et al., 2017). In Nepal, it spreads from 3500 m to the upper treeline at around 4200 m a.s.l. and is often associated with other tree and shrub species, such as *Abies spectabilis* (D. Don) Mirb., *Rhododendron arboreum* Smith, *Juniperus recurva* Buch.-Ham. ex D. Don and *Sorbus microphylla* (Wall. ex. J.D. Hooker) Wenzig (Stainton, 1972). The climate of the region is influenced by the subtropical Asian monsoon, with

over 80% of annual precipitation falling between June and September (Fig. 2). Winters are normally dry, although occasional mid-latitude cyclones can cause heavy snowfall events (Salerno et al., 2015). The temperature data for 1902–2014 collected from CRU TS 3.22 dataset (Harris et al., 2014) indicates that January is the coldest and July the warmest month with a mean temperature of -8.4°C and 9.3°C .

2.2. Tree-ring sampling and analysis

Tree cores were extracted at breast height (1.3 m) with a Pressler borer from 73 mature *B. utilis* trees close to their local altitudinal limit (i.e., between 3850 and 4050 m a.s.l.). Cores were glued on wooden mounts, carefully sanded and ring widths (MRW) were then measured to the nearest 0.01 mm, using LINTAB measurement equipment fitted with a stereoscope and equipped with TSAP software (Frank Rinn, Heidelberg, Germany). Ring-width series were crossdated to match each tree ring with its year of formation and dating was checked with COFECHA software (Holmes, 1983; Stokes and Smiley, 1968). Out of the 73 trees cores, we selected 7 cores (corresponding to 7 trees) for anatomical analysis, avoiding samples with visible faults such as nodes, reaction wood, rotten or missing parts.

2.3. Anatomical analyses

Anatomical analyses were carried out on tree cores. These were split into 4–5 cm long pieces. A rotary microtome (Leica RM 2245, Heidelberg, Germany) was used to obtain 12 μm transversal sections. The samples were stained with Safranin-Astra blue and fixed on permanent slides with Eukitt (BiOptica, Milan, Italy). Digital images were captured with a slide scanner (D-sight 2.0 System, Menarini Diagnostics, Florence, Italy) at 100x magnifications. The images were then processed with the image analysis software ROXAS v.3.0 (von Arx et al., 2016; von Arx and Carrer, 2014). *Betula utilis* wood is composed of fibres, vessels, and scattered apotracheal parenchyma cells irregularly distributed among the fibres (Carlquist, 1988; Morris et al., 2016). Usually, these cells were not automatically recognized and measured by ROXAS because their lumen was filled with residual of cell content. Moreover, considering the low number of this cell type (10–15 cells within a ring); their contribution to the final descriptive statistics can be considered negligible (Fig. 3). In any case, manual editing was performed to remove objects wrongly identified as vessels or fibres. The edited images were then duplicated and for each duplicated image we applied a size threshold to filter out cells smaller than vessels or larger than fibres (Fig. 4). After few trials, this threshold was set at $1000\ \mu\text{m}^2$, i.e., bigger than the widest fibres and smaller than the smallest vessels.

After image editing and filtering, ROXAS automatically calculated several anatomical parameters at the ring level, such as ring width (MRW, mm), plus several anatomical parameters of vessels and fibres separately, i.e. number of cells (Num), cell density (CD, no/mm^2), mean cell lumen area (MCA, μm^2), and mean lumen area of the three largest cells (CAMax3, μm^2). In addition, but just for vessels, ROXAS automatically calculated the mean hydraulic diameter ($D_h = \Sigma d^5 / \Sigma d^4$, where d is the diameter of a given vessel, μm) (Kolb and Sperry, 1999), the xylem-specific hydraulic conductivity (K_s , $\text{m}^2\ \text{s}^{-1}\ \text{Mpa}^{-1}$), computed as the sum of the single vessels' conductivity (k_h , assessed according to Hagen-Poiseuille's law, (Tyree and Zimmermann, 2002) within analyzed xylem area, and the tree-ring specific hydraulic conductivity (K_r , $\text{m}^4\ \text{Mpa}^{-1}\ \text{s}^{-1}$), i.e. the sum of the hydraulic conductivity of all vessels in each ring within an area of fixed tangential width (Castagneri et al., 2015).

2.4. Statistical analysis

To check the quality of the ring-width chronology, we calculated commonly used statistics such as the mean sensitivity (MS) to assess the high-frequency variance; the expressed population signal (EPS), which

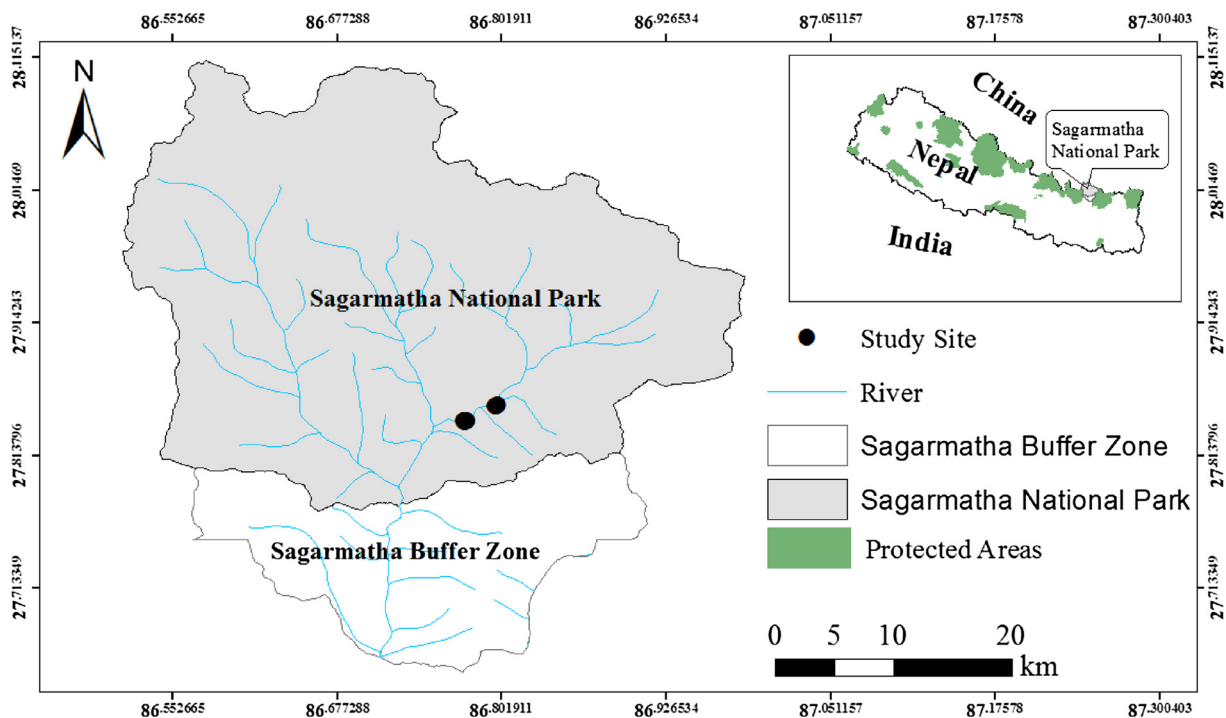


Fig. 1. Map of the Sagarmatha National Park, northeastern Nepal.

is the extent to which the sample size is a representative of a theoretical population with an infinite number of individuals, the first-order autocorrelation (AC(1)); and the mean inter-series correlation (R_{bar}) (Cook and Kalriukstis, 1990; Fritts, 1976; Holmes et al., 1986; Wigley et al., 1984). To remove the size/age trends commonly observed in xylem traits as well as in MRW (Carrer et al., 2015), we detrended all anatomical series with a cubic smoothing spline with 50% frequency cutoff equal to 67% of the series length, using dplR R package (Bunn, 2008). The detrended series of 7 trees were averaged by a bi-weight robust mean (Cook, 1985) to build the mean anatomical chronologies. Principal component analysis (PCA) was used as a data-reduction technique to assess the relationships within the various anatomical parameters and to condense them to a minimum set of variables with the maximum amount of differing information. When two or more variables presented similar loadings for the first two principal components, the one with the largest loadings has been selected for successive analyses to avoid redundancy.

We assessed the climate effects on the MRW and anatomical traits by using the CRU TS 3.22 monthly temperature and precipitation dataset from 1902 to 2007. To verify the representativeness of CRU dataset for our study area we correlated, for the 1994–2012 period, precipitation and temperature series with corresponding records from a nearby meteorological station (Pyramid Laboratory, 5050 m). Correlation with bootstrapped confidence intervals (Biondi and Waikul, 2004) was calculated between MRW and anatomical chronologies and monthly climate data. As the climate in the preceding growing season often influences tree growth during the following year (Fritts, 1976), we considered temperature and precipitation data from July of the previous year to September of the current year.

3. Results

The high-resolution images allowed distinct annual growth rings boundaries to be identified, made of 2–4 rows of radially flattened parenchyma cells (Fig. 4). As a typical feature for a diffuse-porous species, vessels were sparsely scattered within the tree rings (Supplementary Data, Fig. S1). Analysis between the nearby meteorological

station and CRU gridded dataset TS3.22 showed a strong correlation between temperature data ($r = 0.95$) and weaker but significant correlation between precipitation data ($r = 0.79$) (Supplementary Data, Fig. S2). We, therefore, used the CRU gridded dataset of mean monthly temperature and precipitation for the period as used by other researchers where meteorological stations are lacking (Sohar et al., 2016).

We built up a ring width (MRW) chronology spanning from 1610 to 2007 with the individual series length varying from 91 to 397 years. The MRW series of all samples showed rather high values of mean sensitivity, mean correlation between trees and an expressed population signal (Table 1), suggesting adequate replication and high common signal shared by trees. However, when calculations were restricted to the 7 cores used for the anatomical analyses, MRW series showed lower values (Supplementary Data, Table S1). Likewise, the same statistical parameters calculated for the vessels and fibres were also rather low (Supplementary Data, Table S1).

3.1. Inter-correlations between anatomical traits of tree rings

Vessel and fibre traits showed complex inter-relationships (Fig. 5). Explained variance by the first two principal components in the PCA was 41.91 and 24.52% (66.43% in total). This analysis highlighted the existence of highly correlated variables (Fig. 5), namely MRW and Num_V; Dh_V and MCA_V, CAMax3_F and MCA_F. For each of these pairs, we selected the variable with the highest loading for the following climate association analyses.

3.2. Climate association of xylem anatomical traits

Climate-growth relationships were assessed only from 1902 to 2007 because of availability of meteorological data using CRU gridded dataset TS3.22. MRW showed significant positive correlations with the current year mean temperature of July, August, September, and of the monsoon season (Fig. 6), whereas previous year July and September temperature also had a significant positive effect on MRW. In addition, significant positive correlations occurred with precipitation in previous

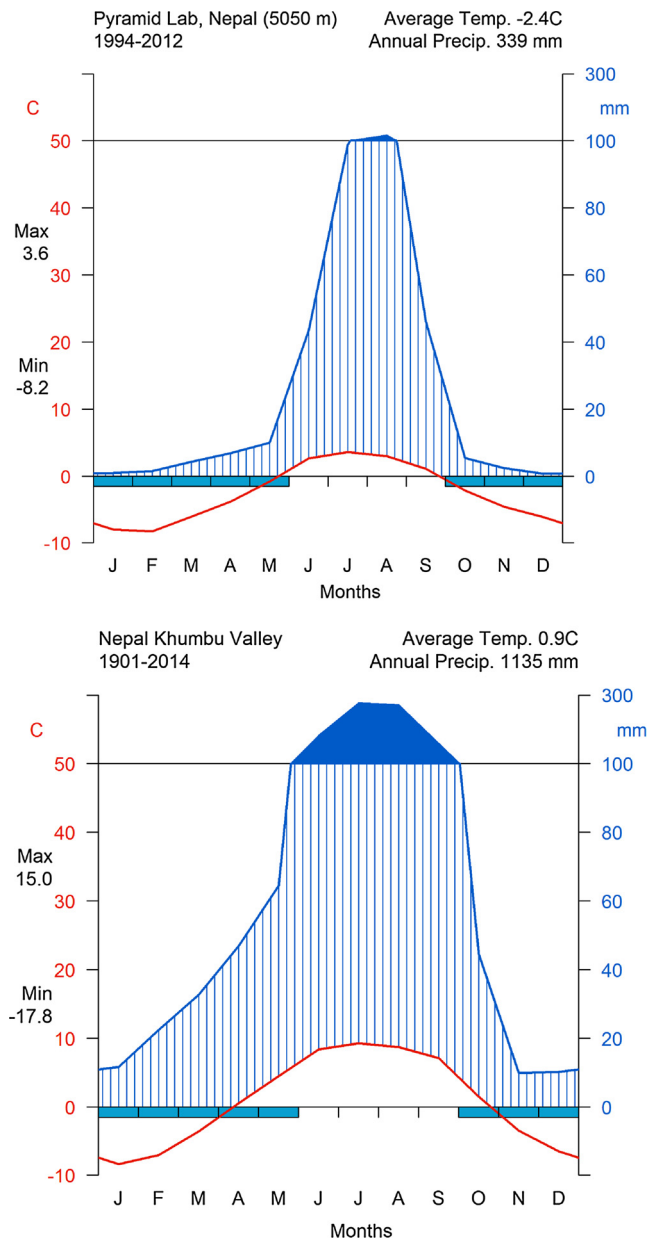


Fig. 2. Walter-Lieth ombrothermic diagram for Pyramid Laboratory, Lobuche, Sagarmatha National Park (5050 m) and for Climate Research Unit's grid climatic data with 27.97°N latitude and 86.66°E longitude, Nepal Khumbu Valley the *B. utilis* sample site.

October and December and with pre-monsoon period (March, April, May) (Fig. 7).

The anatomical parameters of vessels showed significant correlations with climate parameters (Figs. 6 and 7). Vessel-related traits, such as MCA_V, CAMax3_V and Ks_V showed a positive correlation with mean temperatures in the previous year August and current year June and August. MCA_V and CAMax3_V had a positive correlation with monsoon (JJAS) temperature. CD_V showed a negative correlation with previous year July and August and current year July, August, September and JJAS temperature. Kr showed a significant correlation with July, August and September temperature of the previous and current year. The JJAS temperature had a significant positive correlation with Kr. There was a positive correlation between MCA_V and pre-monsoon (MAM) precipitation, and that of the current year March in particular. Previous year December and current year April and MAM precipitation showed a significant negative correlation with CD_V. Kr showed a

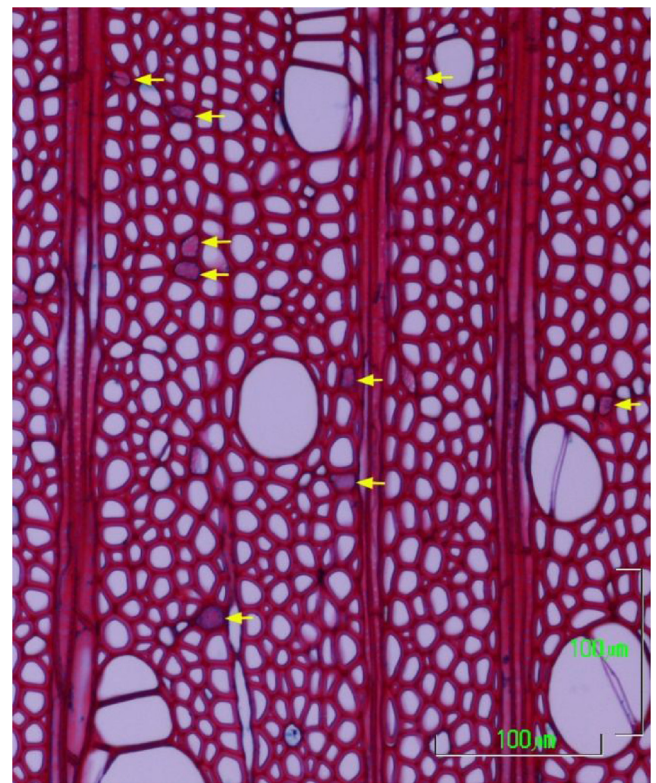


Fig. 3. Example of *Betula utilis* cross-section taken at 100× magnification. Arrows indicate the apotracheal parenchyma cells, recognized based on the presence of residual blue-stained cell content.

positive correlation with previous year October and current year February, April and MAM precipitation, while Ks_V did not show significant correlation with precipitation at any time of current and previous year.

The size parameters of fibres showed significant correlations with temperature, but not with precipitation (Figs. 6 and 7). MCA_F was negatively correlated with previous year August to December and current year January, February, April, May, June, August and MAM temperature. Seemingly, CAMax3_F showed a negative correlation with previous October and current May and MAM temperature. CD_F also showed a negative correlation with previous year August, November, December and current year February, March and MAM temperature.

4. Discussion

This study reported the association of monthly temperatures and precipitations with different xylem anatomical traits in *Betula utilis* growing close to the world's highest tree line. Our aim was to explore whether the different traits encode significant and distinctive climate signals that reveal the ecological behaviour of Himalayan birch in one of the world's climate change hotspots.

The results highlighted that tree growth responses are consistent with the typical Himalayan climate dominated by the monsoon seasonality, characterized by cold dry winters, and summer with abundant precipitation and daily temperature above 10 °C (Neupane et al., 2015; Thakuri et al., 2014). The climate signal encoded in our Himalayan birch trees was significant (mean sensitivity (MS) = 0.22, series inter-correlation (Rbar) = 0.24) and in agreement with previous research (Dawadi et al., 2013). The chronology statistics are lower than those of Himalayan birch in western Himalaya (Bhattacharyya et al., 2006) and higher than *B. ermanii* in Changbai Mountains, northeast China (Yu et al., 2005). Forest vegetation in these areas has already been reported to be sensitive to the environmental conditions in early spring

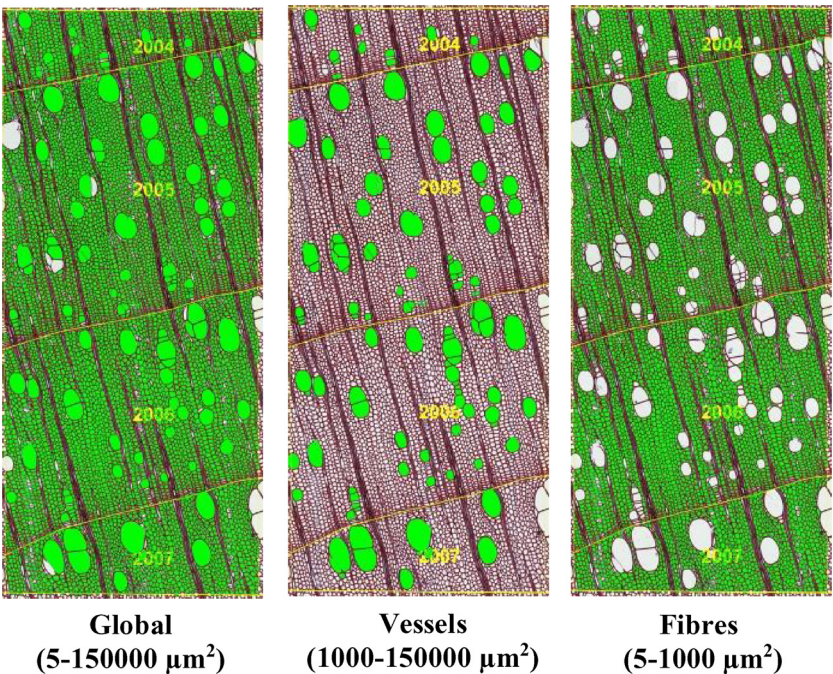


Fig. 4. Image of a cross-section of *Betula utilis*. Size filtering was used to separate vessels (above 1000 μm^2) and fibres (below 1000 μm^2).

Table 1
Selected statistics of the standard tree-ring width (MRW) chronology.

Parameters	Duration/value
Chronology time span (year)	1610–2007 (397)
Numbers of trees	73
Mean	0.96
Standard deviation	0.26
Mean sensitivity (MS)	0.22
Expressed population signals (EPS)	0.88
First-order correlation (AC(1))	0.52
Series inter-correlation (Rbar)	0.24

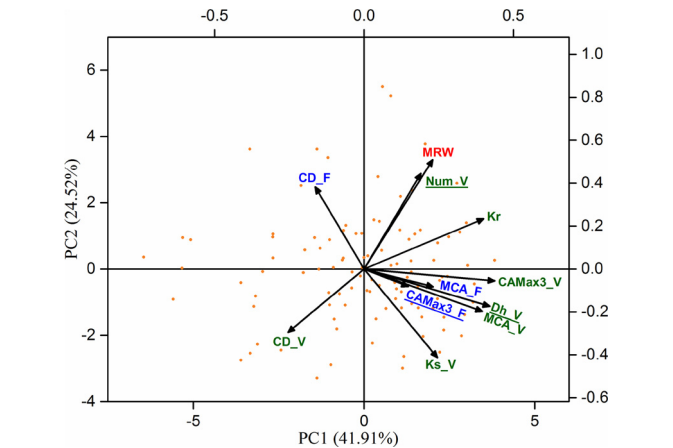
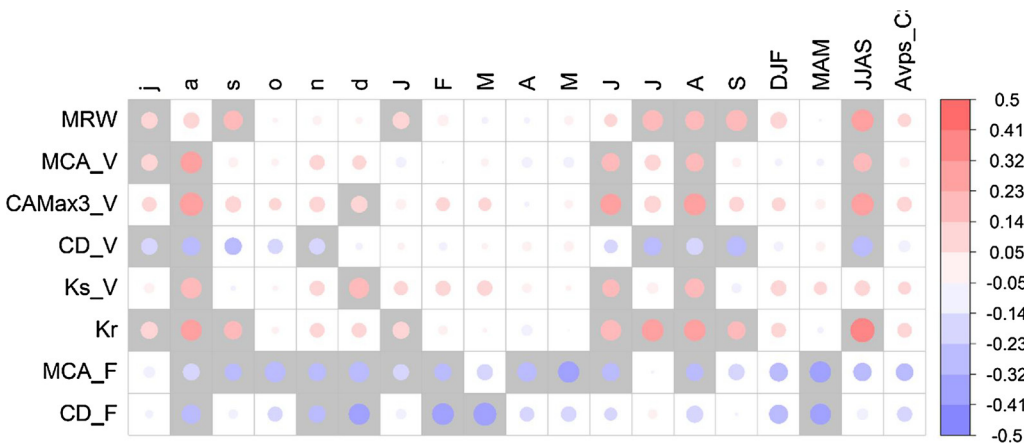


Fig. 5. Biplot of the principal component analysis of mean ring width (MRW, in red) and anatomical traits of vessels (indicated by V, in green) and fibres (indicated by F in blue). Xylem anatomical traits are: mean cell area (MCA), mean area of the three largest cells (CAMax3), mean hydraulic diameter (Dh, assessed only for vessels), number of cells (Num), cell density (CD). Underlined variables were removed from further analyses (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

(Bhattacharyya et al., 2006; Dawadi et al., 2013; Gaire et al., 2015), especially at higher elevations. The classical estimators (mean sensitivity (MS), series inter-correlation (Rbar), and expressed population signals (EPS)) for chronology were weak for the vessel and fibre, with values close to zero or even negative for fibre. This is common in chronologies based on anatomical features (Carrer et al., 2017, 2016; Fonti and García-González, 2004; Olano et al., 2013) and has been attributed to either a lower inter-annual variation in anatomical parameters owing to functional constraints or to the reduced area examined in anatomical studies, as they are commonly based on microscopic preparations (García-González and Fonti, 2008).

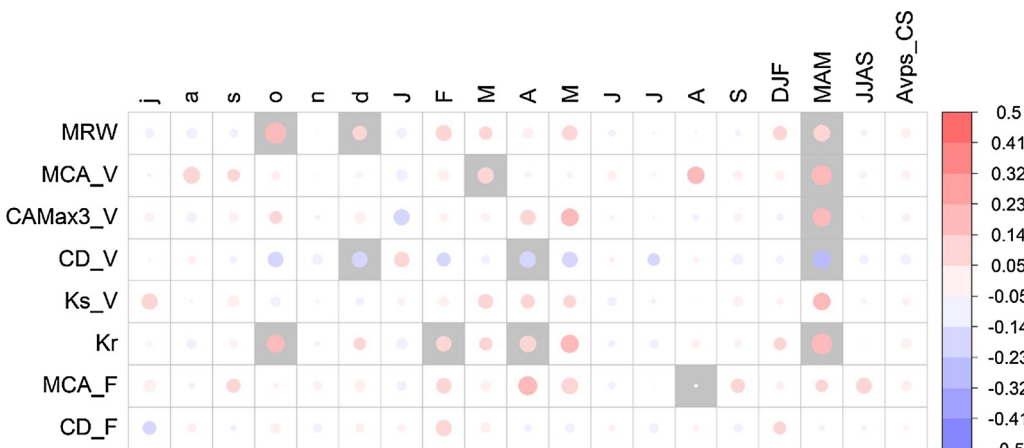
The tree's secondary growth (i.e., ring width) typically responds to early season droughts with a positive correlation with precipitation and a negative correlation with temperatures (Dawadi et al., 2013). Instead, previous investigations most commonly found reverse associations with the summer season, i.e., negative and positive correlations for precipitation (Fritts, 1976; Urban et al., 2012) and temperature (Chaves et al., 2002; Lovisolo and Schubert, 1998), respectively.

Our results revealed that tree growth (i.e., mean ring width: MRW) and xylem hydraulic efficiency (expressed as ring-specific hydraulic conductivity: Kr) benefit from high temperatures during the monsoon season of the previous and current summer. We can thus hypothesize that carbon reserves stored during the previous summer can play a role in sustaining the investment in new tissues at the beginning of the growing season (Klein et al., 2016). In addition, the results suggested that cambial activity continued to be stimulated by higher temperatures during the summer either because of the higher photosynthetic potential with a clear sky or because of the release from cold temperature limitations to enzymatic fixation of carbon into biomass (Körner, 2012; Rossi et al., 2007). Indeed, correlations of mean cell area of vessel (MCA_V) and mean area of the three largest cells of vessel (CAMax3_V) with previous and current summer temperatures suggest that formation of wide earlywood vessels relies upon carbon reserves stored during the previous summer, whereas large vessels across the ring are determined by the better environmental conditions related to high atmospheric temperatures. The corresponding negative correlation between vessel density (CD_V) and previous and current summer temperatures is consistent with this, because of the geometrical constraint that only a lower number of wider vessels can fit a given surface area, irrespective



according to circle's size and colour ($-0.5 < r < 0.5$), and grey background indicates significant correlation.

Fig. 6. Monthly and seasonal correlation between mean ring width (MRW) and anatomical traits of vessels (indicated by V) and fibres (indicated by F) with temperature. Xylem anatomical traits are: mean cell area (MCA), mean area of the three largest cells (CAMax3), cell density (CD). Hydraulically related vessel traits are xylem-specific hydraulic conductivity (Ks) and tree-ring-specific hydraulic conductivity (Kr). Period spans from July of the previous year to September of the current year. DJF, MAM, JJAS, and Avps_CS represent winter, pre-monsoon, monsoon and the yearly mean from previous to current September respectively. Correlation coefficients are coded according to circle's size and colour ($-0.5 < r < 0.5$), and grey background indicates significant correlation.



circle's size and colour ($-0.5 < r < 0.5$), and grey background indicates significant correlation.

Fig. 7. Monthly and seasonal correlation between mean ring width (MRW) and anatomical traits of vessels (indicated by V) and fibres (indicated by F) with precipitation. Xylem anatomical traits are: mean cell area (MCA), mean area of the three largest cells (CAMax3), cell density (CD). Hydraulically related vessel traits are xylem-specific hydraulic conductivity (Ks) and tree-ring-specific hydraulic conductivity (Kr). Period spans from July of the previous year to September of the current year. DJF, MAM, JJAS, and Avps_CS represent winter, pre-monsoon, monsoon and the yearly mean from previous to current September respectively. Correlation coefficients are coded according to circle's size and colour ($-0.5 < r < 0.5$), and grey background indicates significant correlation.

of fibres' geometry (i.e., the so-called packing constraint) (Savage et al., 2010).

Concerning the effects of precipitations, the various dendro-anatomical traits indicated that water availability is limiting only during the pre-monsoon period. The earliest positive correlations were found for mean ring width (MRW) and ring-specific hydraulic conductivity (Kr) with previous October's precipitations, which can occur as snow at the study site. This can provide an important water source when the snow melts the following spring (Shrestha et al., 2007). Late season precipitations would also increase the capacity to fully replenish water reserves in the storage tissues (Köcher et al., 2013; Turcotte et al., 2011). Interestingly, average vessel area (MCA_V) was positively correlated with spring precipitations, but not with those in the previous year. We hypothesize that precipitation from the previous year monsoon, together with the current year early spring ones, stimulates the rate of cambial activity after the winter break, and induces the necessary turgor pressure to distend the first rows of earlywood vessels (González and Eckstein, 2003). As a result, while ring-specific hydraulic conductivity (Kr) is positively correlated with precipitations in the previous October and current spring, the overall mean ring width (MRW) is not affected by spring precipitations, suggesting that this latter parameter is more affected by the climatic conditions occurring across the entire growing season.

We found that fibres encoded a weaker climate signal than the other anatomical variables. The average fibres' lumen area (MCA_F) was negatively correlated with temperatures, thus showing an opposite response than vessels and implying that fibres are narrower when vessels are wider. This contrasting behaviour of these two anatomical traits

could underline a coordinated plastic anatomical adjustment aimed at assuring a certain degree of hydraulic safety (i.e., narrower fibres) also for a higher investment in xylem conductivity (i.e., larger vessels). Indeed, narrower fibres were reported to confer a higher resistance against embolism formation to the whole xylem structure (Jacobsen et al., 2005), as well as a higher resistance to mechanical stress (Zanne et al., 2010).

Our analyses conducted on fibre anatomy provide the first evidence that, due to recent advances in anatomical sample preparation (Castagneri et al., 2015) and image analysis of tree cores (von Arx et al., 2016), it is now technically possible to build up long time series of this understudied anatomical trait. In this respect, it is important to underline that other studies are needed to more clearly determine the potential value of fibre anatomy in dendrochronological studies.

5. Conclusion

The anatomical features of the plants' xylem at the stem base are mainly determined by genetic factors (Vaganov et al., 2006) and tree height (Carrer et al., 2015; Olson et al., 2014), but a large part of inter- and intra-annual variability is driven by climatic and environmental processes to which trees respond by adjusting their xylem structure (Fonti and Jansen, 2012; Wimmer, 2002). We found that Himalayan birch at one of the world's highest treelines in Eastern Nepal seemed to adjust xylem features to inter-annual climate variations influenced by the monsoon seasonality. As for most dominant vegetation in cold environments, this species also appeared to be most sensitive to the temperature regimes during the growing season. Since temperature

limits xylem formation at the treeline, relaxation of this limiting factor by continuous climate warming will likely stimulate the further expansion of *B. utilis* to higher elevations in Eastern Nepal.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ppees.2018.05.004>.

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